Abstract

Bacteria living in the gastrointestinal (GI) tract are crucial for human health and disease occurrence. Increasing the beneficial intestinal microflora by consumption of *prebiotics,* which are "*functional foods"*, could be an elegant way to limit the number and incidence of disorders and to recover from dysbiosis or antibiotic treatments. This review focuses on the short-chain low-digestible carbohydrates (LDCs) which are metabolized by gut microbiota serving as energy source, immune system enhancers or facilitators of mineral uptake. Intake of foods containing LDCs can improve the state of health and may prevent diseases as for example certain forms of cancer. Given the large number of different molecules belonging to LDCs, we focused our attention on fructans (inulin, fructo-oligosaccharides: FOS), galacto-oligosaccharides (GOS) and resistant starches and their therapeutic and protective applications. Evidence is accumulating that LDCs can inhibit bacterial and viral infections by modulating host defense responses and by changing the interactions between pathogenic and beneficial bacteria. Animal studies and studies on small groups of human subjects suggest that LDCs might help to counteract colorectal cancer, diabetes and metabolic syndrome. The action mechanisms of LDCs in the human body might be broader than originally thought, perhaps also including reactive oxygen species (ROS) scavenging and signaling events.

Key words: prebiotics, low-digestible carbohydrates, fructans, colon cancer prevention, gastro-intestinal diseases.

Table of content:

Introduction

Nowadays an increasing number of people try to prevent diseases and reach a better health by modifying their eating habits and life style. According to the World Health Organization (WHO, 1948) "*Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity*".

It is well known that the intake of certain foods can improve the state of health and might prevent some diseases as for example certain forms of cancer which frequently affect the population. In this context we can easily understand the importance, not only from a medical but even from a commercial perspective, of the so-called "F*unctional foods*" defined as "*Natural or processed foods that contain known biologically-active compounds which, when dosed in defined quantitative and qualitative amounts, provides a clinically proven and documented health benefit, and thus, an important source in the prevention, management and treatment of chronic diseases of the modern age"* (Martirosyan, 2011). This review focuses on low-digestible carbohydrates (LDCs), which may be used in prevention of many diseases and especially colorectal cancer.

Life inside the intestine

The peculiar structural characteristics found in the intestinal tract of all mammals and the presence of metabolites that can be used as substrates by microorganisms make it a suitable environment for the colonization and the development of a diverse microbial flora. So far, approximately 400 different

microbial species have been identified (Frank and Pace, 2008) and reported from different zones of the intestinal tract from healthy individuals (Fig. 1).

More specifically, we can assert that the class of *Bacilli*, *Firmicutes* and *Actinobacteria* are prevalent in the small intestine and *Bacteroidetes* as well as *Lachnospiraceae* are typical in the colon (Frank *et al.*, 2007). Furthermore the density of microorganisms varies along the entire digestive tract ranging from 10-1,000 CFU/ml in the stomach to 10-100 billion CFU/g in the large intestine, since it constitutes an appropriate habitat for the development of such symbionts (Farthing, 2004).

The intestinal epithelium presents a superimposition of the internal muscle tissue surrounded by the mucous membrane which presents microvillosities and also a physic-chemically complex mucus layer that separates it from the intestinal lumen. This structure enables the creation of additional micro-environments suitable for the development of different microbial species. Typically, *Clostridium*, *Lactobacillus* and *Enterococcus* were observed in the mucus coating and epithelial crypts of the small intestine while *Bacteroides*, *Bifidobacterium*, *Streptococcus*, *Enterococcus*, *Clostridium*, *Lactobacillus* and *Ruminococcus* were detected in feces (Sekirov *et al.*, 2010). Furthermore, Wang *et al.* (2010) demonstrated region-specific gene expressions in the epithelium of the colon-mucosa and this could be linked to the presence of specific microbiota.

Anaerobic flora is dominated by Bacteroides spp., Bifidobacteria, Propionibacteria and Clostridia. Among aerobic and anaerobic bacteria Enterobacteria, mainly E*scherichia* coli, and Enterococci predominate (Swidsinski *et al*., 2005). The intestinal microflora is not homogeneous among the population but greatly varies between people. Moreover, within a certain individual it is subject to extensive variation during the course of life. The central role of the intestinal microflora in health and disease incidence has been recently revalued after the renewed interest in their structure and function. The gut symbiotic microorganisms are closely related to different aspects of regular host physiology, ranging from the introduction, absorption and utilization of nutrients to the lifestyle and stress responses. Accordingly, many diseases, which are not necessarily located at the intestinal level, can be triggered or influenced by a disturbed gut microflora since this equilibrium can be easily modified by many factors (e.g. diet, climate, aging, use of medication, illness and lifestyle) (Correia and Nicoli, 2006). The flexible character of the intestinal microflora, due to their continuous activity and proliferation, is an essential feature allowing them to act as a natural barrier not only capable of performing structural and metabolic functions, but also able to protect the intestinal wall. Their overall functionality can be summarized into three major tasks which are colonization resistance to pathogens; modulation of gastrointestinal and systemic immune responses and nutritional support (Olmstead *et al*., 2008).

Through the production of short-chain fatty acids (SCFAs), resident bacteria provide energy and nutrients for their growth, absorbable substrates for the host and positively influence the cell cycle of intestinal epithelium as well as other metabolic effects (Shanahan, 2002).

In healthy individuals microbiota can fulfill direct defensive functions through the direct impediment of the colonization by pathogens competing for

space and nutrients or indirectly by the production of antimicrobial compounds, volatile fatty acids, and chemically modified bile acids. In this way, through the colonization resistance or barrier effect, the indigenous gut bacteria are able to establish adverse conditions for the inoculation and/or development of the enteric pathogens (Roderick et al., 1999).

In general, if there is a prevalence of gram-positive bacteria *Lactobacilli* and *Bifidobacteria* in the gut, a condition termed *eubiosis* is established. This term indicates a condition of equilibrium between the host and all the intestinal microbial population, instead a disordered state in these symbiotic gut inhabitants is known as *dysbiosis.*

Prebiotics: Gut Microflora "energizing molecules"

A well-known strategy allows sustaining the activity of intestinal microflora by the regular intake of beneficial probiotic organisms. Another approach is to utilize "prebiotics" to stimulate beneficial bacteria to restore from dysbiosis or antibiotic treatments. In contrast to the probiotic strategy which provides living microorganisms, the prebiotic application is able to stimulate the life cycle and/or increase the metabolic activity of healthful bacteria that are already inhabitants of the intestine. For that reason, prebiotics

are considered simpler and more effective modulators of the gut microflora compared to probiotics.

In the ‘50s György *et al*. (1953-1954) reported that the consumption of substances associated with the diet had positive effects on health by acting on the growth and development of *Bifidobacteria* (known as *Lactobacillus bifidus* at that time) and therefore he termed this the *bifidus factor*. Later in the ’70s and ’80s similar studies on the use of digestion-resistant saccharides like fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS) and lactulose were undertaken by Japanese researchers. Also in this case the results indicated that these compounds had a beneficial effect on the intestinal microflora (Yazawa *et al*., 1978) and some of these products came on the market in the ’80s and ’90s. Only in 1995, Gibson and Roberfroid gave, for the first time, an exhaustive description of prebiotics and their functionalities. Recently prebiotics have been redefined as selectively fermented dietary components permitting precise variations in the composition and/or activity of the gastrointestinal microflora and beneficial for the host health (Roberfroid, 2007a).

Prebiotics are “*functional foods”* and in compliance with the European Consensus on ‘Scientific Concepts of Functional Foods: “*A food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either improved stage of health and well-being and/or reduction of risk of disease”* (Diplock *et al*., 1999).

Furthermore a food can be considered as functional only if it performs health benefits in doses which can be normally consumed in a diet and as a normal form. Therefore, pills and capsules cannot be considered as functional foods.

Low-digestible carbohydrates (LDCs), are not (or minimally) digested in the stomach or small intestine and are carried on into the large intestine where they are fermented by gut microbiota (Scheppach *et al.*, 2001; Holzapfel and Schillinger, 2002; Grabitske and Slavin, 2008). Some of the metabolites produced in this way can be used as energy source, immune system enhancers, facilitators of mineral uptake, etc. (Holzapfel and Schillinger, 2002). LDCs selectively stimulate beneficial bacterial groups residing in the colon such as *Bifidobacteria*, *Lactobacilli* and *Eubacteria* species (Livesey, 2001; Holzapfel and Schillinger, 2002). In addition, LDCs may constrain colonization of pathogenic bacteria such as *Clostridium* and *Bacteroides* (Holzapfel and Schillinger, 2002). Additional benefits associated with LDC consumption may include prevention of diseases such as:

- Type II diabetes (by regulating levels of plasma glucose, insulin secretion and insulin sensitivity)
- Coronary heart disease (by modulating plasma lipid concentration)
- Gastrointestinal diseases such as colon cancer (by colon acidification, stool bulking, protection from pathogen colonization and modulation of bacterial microflora)
- Dental cavities (inhibition of oral acidification)
- Obesity (low energy value and promotion of satiety)

Although there are numerous potential benefits associated with consumption of LDCs, some dose-related undesirable effects may also occur. Unwanted symptoms are usually linked to excessive fermentation and/or osmotic effect of prebiotics, including e.g. excessive flatus, bloating, abdominal cramps, loss of appetite, sweating, thirst and diarrhea (Livesey, 2001; Marteau and Flourié, 2001; Holzapfel and Schillinger, 2002; Grabitske and Slavin, 2009). Severity of symptoms is highly dependent on the administrated dosage (Marteau and Flourié, 2001; Grabitske and Slavin, 2009). Tolerance of LDCs is influenced by many factors from which ones mentioned below are the most important:

- Gender – in woman LDCs are usually retained in the upper gastrointestinal tract which may cause symptoms as nausea and to a slighter extent laxation (Buemann *et al*., 1999)
- Age – due to the immature gastrointestinal tract in infants and adolescent children diarrhea is up to six time more common than in adults (Payne *et al*., 1997)
- Molecular weight – LDCs exert an osmotic effect in the intestinal lumen which is negatively related to their molecular weight. The increase of water flow rate may induce borborygmi, abdominal pain, and diarrhea; especially if the capacity of the colon to absorb water and electrolytes is exceeded (Marteau and Flourié, 2001)
- Composition of colonic flora - regular consumption of LDCs results in changes in the metabolic activity of the colonic flora and increases its fermenting ability. Fewer diarrheas might be expected when LDCs are consumed regularly (Florent *et al*., 1985; Marteau and Flourié, 2001)

- Gastrointestinal motility and transit time – LDCs which are not fermented are excreted, triggering an increase of bulk and stools water content. If transit time or GI motility is too poor for the intestinal cells to absorb the excess water, this may induce diarrhea (Grabitske and Slavin, 2008).

Clinical research studies reported on the toleration doses for various LDCs. Non-starch carbohydrates like guar gum, inulin, and FOS are in general well-tolerated at intake levels up to 15 g/day (Grabitske and Slavin, 2009). Polydextrose is tolerated at much higher doses of maximum 50 g/day (Flood *et al*., 2004) and resistant starch is well-tolerated at doses not exceeding 45 g/day (Van den Heuvel *et al*., 2004; Robertson *et al*., 2005). Assessed acceptable intake of sugar alcohols ranges from 20 g/day to 40 g/day (Rushton and Slavin, 2007). Since dose-related intolerance symptoms may occur during the treatment, it is important to adjust the therapeutic dose to each individual patient. The therapeutic window is frequently wide-ranging and preeminent benefits can be obtained with accurate dosage (Delzenne, 2003).

Fructans, substances with a proven prebiotic effect

Fructans are a group of non-structural carbohydrates occurring in 15% of the flowering plant species and in some bacteria, algae and mosses (Hendry and Wallace, 1993). The identification of the first fructan has occurred in the 1804 by a German scientist who found "*a peculiar substance from plant origin in a boiling water extract from Inula helenium".* A few years later, in 1818, another researcher, Thompson, identified this substance as inulin.

Inulin-type fructans are synthesized and stored in vacuoles of Asteracean plants. Particular species belonging to this family play an important economic and nutritional role such as chicory (*Cichorium intybus*) Jerusalem artichoke (*Helianthus tuberosus*) (Carabin and Flamm, 1999) and artichoke (*Cynara*) accessions (Raccuia and Melilli, 2010).

Fructans are polymers of fructose that often contain a terminal glucose moiety. They can have a linear or branched structure. For simplicity, a subdivision into five main groups can be made on the basis of the position of glucose moieties (internal or end position) and on the glycosidic linkages between their fructosyl residues (Fig. 2). Inulin-type fructans consist of fructose units linked through β-2,1 bonds and displaying a linear structure. 1-kestotriose (1-kestose) is the simplest inulin-type fructan produced by the activity of a sucrose:sucrose 1-fructosyl transferase (1-SST) which transfers a fructosyl residue from donor to an acceptor sucrose, producing 1-kestotriose. Fructan:fructan 1-fructosyl transferase (1-FFT) further polymerizes 1-kestotriose into higher DP inulin-type fructans (Fig. 2). Sucrose:fructan 6-fructosyl transferase (6-SFT) preferentially transfers a fructosyl group from sucrose as a donor substrate to 1-kestotriose as acceptor substrate, producing 1&6-kestotetraose (also termed bifurcose), the smallest graminan-type of fructan with mixed type of linkages (Fig. 2). Bifurcose can be further elongated by 6-SFT and 1-FFT, leading to branched, higher DP graminans. The levan-

type fructans consist of β-2,6 fructosyl–fructose bonds. They are linear and 6-kestotriose (6-kestose) is the shortest fructan of the levan-type. Levan synthesis is believed to occur by a sucrose:fructan 6-fructosyl transferase with intrinsic sucrose:sucrose 6-fructosyl transferase characteristics (a 6-SST/6-SFT). Finally, the enzyme fructan:fructan 6G-fructosyl transferase (6G-FFT) synthesizes 6G-kestotriose (6G-kestose, neokestose) from 1-kestotriose as donor substrate and sucrose as acceptor substrate. Further elongation by 1-FFT and 6-SFT leads to the formation of inulin neoseries and levan neoseries, respectively (Van den Ende *et al.*, 2002; Lasseur *et al.*, 2006; Tamura *et al.*, 2009).

A common way to denote, in an abbreviated form, the structure of fructans is indicating the glucose units with G and the number of fructose units with Fn. The first monomer that starts the fructan chain may be a β-D-glucopyranosyl, as in GpyFn [glucopyranosyl-(fructofuransoyl) n-fructose] or a β-D-fructopyranosyl residue as for example in FpyFn [fructopyranosyl-(fructofuranosyl) n-fructose] (Vester-Boler and Fahey, 2012).

Oligosaccharides and microbiota

Oligosaccharides are low molecular weight carbohydrates with a degree of polymerization (DP) ranging between 2 and 9. They are easily soluble in water and their typical sweetness decreases with their increasing chain length.

On the contrary, their water-binding and gelling properties, which make them putative fat substitutes, increase with the number of hexose molecules. The principal sources of these substances are chicory, asparagus, artichoke, onions, garlic, leeks and soya beans as well as in human breast milk and cow's milk (Conway, 2001). The pivotal characteristic of oligosaccharides, once eaten, is their resistance to be metabolized by hydrolytic enzymes as α-glucosidase, maltase and isomaltase secreted into or active in the intestine. The digestive capacity of these enzymes towards β-2,1 fructans (inulins) is ineffective. Digestibility can also greatly depend on their DP. For instance, isomalto-oligosaccharides with a DP > 3 cannot be properly digested (Delzenne, 2003).

Oligosaccharides, which mostly escape digestion in the upper gastrointestinal tract, are important sources of energy for bacteria in the caeco-colon that express enzymes such as β-fructosidase, β-galactosidase, xylanase or other hydrolases (Bernalier *et al*., 1999). The glycosidic bonds arrive in the colon almost intact (with the exception of some slight hydrolysis in the stomach) because of their resistance to enzymatic hydrolysis in the previous part of the intestine. The process of fermentation operated by colonic microbes on LDCs generates SCFAs as main products and also gases such as carbon dioxide, methane and hydrogen. An important outcome is the pattern of fermentation, i.e. the proportion of the different SCFAs acetate, propionate, butyrate and lactate, produced in the caecum varies with the nature of the oligosaccharides, at least in animals. The ratio between the level of acetate and propionate is six-times higher in the caecum of rats fed with a diet based on GOS compared with a control population whose diet contained the same quantity of FOS (Sakaguchi *et al*., 1998). The proportion also changes with the duration of the treatment; for example a study showed that in rats, whose diet was enriched in FOS, a temporal increase in lactate and a constant increase in

butyrate was observed after 27 weeks (Le Blay *et al.*, 1999). A diet rich in FOS also led to elevated sulfomucins in the caeco-colonic mucosa of rats. These compounds are known for their protective activity (Cherbut, 2002). The SCFAs generate important effects in the intestinal tract. It is broadly accepted that butyrate fulfills an important function in maintaining the metabolism, proliferation and differentiation of the different epithelial cell types (Blottiére *et al.*, 1999). Unfortunately, since there is not a direct correspondence between the real production of SCFAs in the colon and the quantity excreted in the feces, it is not easy to have a clear idea of the effects of prebiotics on the production of SCFAs in humans.

As claimed before, prebiotics can selectively stimulate the growth and/or the activity of selected intestinal bacteria causing changes in the overall microbial population which are health promoting for the host (Gibson, 2008). Is not clear yet which specific mechanisms are implicated in the metabolism of prebiotics, although there are two general models that can partially explain this process. The most accepted model is based on the action of cell-associated exo-glycosidases by probiotic microorganisms (Perrin *et al.*, 2001). Such enzymes act by cleavage of monosaccharides from the non-reducing end of the oligosaccharides, which are then taken up within gut epithelial cells. Furthermore, Kaplan and Hutkins (2000) and Gopal *et al.* (2001) assert that

probiotic microorganisms can perform intracellular metabolism after absorption of the oligosaccharides.

Most of the initial studies on colonic microbiota have focused exclusively on *Bifidobacteria* species present in the fecal material. However, increasing evidence suggests that the epithelial surface is populated by large and diverse bacterial communities, which are completely different from those occurring in the lumen of the intestine (Macfarlane *et al*., 2004). Such bacteria are able to grow in bio-films in the vicinity of the colonic mucosa and they are considered important modulators of immune responses (Neish *et al*., 2000).

A mixture of compounds with specific protective functions, such as the mucins (MUC1, 2, 3, and 4) and trefoil factors (TFF 1, 2, and 3) are present in the mucus bio-film adjacent to the colon. Feeding studies on rats with FOS and resistant starch showed MUC2 increases in the colon, but the effect was superior upon combining both substances (Rodriguez-Cabezas *et al*., 2010). Other studies report increased levels of *Lactobacilli* associated with prebiotic consumption. A significant dose-related increment in fecal *Lactobacilli* was observed in subjects consuming GOS for 7 days (Ito *et al*., 1990). Nevertheless it is critical to take into account the pre-treatment levels of these microorganisms. For example, if there are already high levels of *Bifidobacteria*, it is difficult to show an eventual elevation following the administration of one or more prebiotic(s) (Roberfroid *et al*., 1998).

Currently, the most studied and known prebiotics are GOS, lactulose, inulins and FOS, which can be produced either by extraction from plants or as products of enzymatic activity. Several studies conducted both on animals and on humans (Bouhnik *et al*., 2004; Gibson *et al*., 2004; Roberfroid *et al*., 2010) have confirmed the hypothesis that only limited daily amounts of these substances are needed, ranging from 5 to 20 g, to achieve real beneficial effects

on intestinal microbial populations. It has been demonstrated that symbiotic bacteria, once they get in contact with the prebiotic molecules, are able to metabolize them directly (Ohtsuka *et al.*, 1989). Alternatively, their growth can be indirectly influenced by molecules such as lactate, which are intermediate products from other fermentations leading to a process called cross-feeding (Belenguer *et al.*, 2006; De Vuyst and Leroy, 2011).

Additional molecules which have been recently classified as prebiotics are lactitol, mannitol, maltodextrin, raffinose, lactulose, sorbitol, isomaltooligosaccharides (IMO), mannan-oligosaccharides (MOS) and xylooligosaccharides (XOS). Also wheat bran-derived arabinoxylooligosaccharides (AXOS) are putative promising prebiotics (Sabater-Molina *et al.*, 2009; Femia *et al.*, 2010; Vamanu and Vamanu, 2010; Yeo and Liong, 2010; Broekaert *et al.*, 2011; Corrigan *et al.*, 2011).

Inulin, FOS and GOS

Inulin-type fructans are the most studied and widely applied prebiotics. Inulin molecules with a degree of polymerization ranging from 2 to ≥60 are commercially available (Roberfroid, 2007b) and acknowledged as stimulators of SCFAs in the colon, favoring the growth of *Lactobacilli* and *Bifidobacteria,* associated with reduced mucosal inflammation and lesion scores in a rat model

of inflammatory bowel disease (IBD) (Videla *et al*., 2001). The β-configuration of their bonds makes inulin-type fructans resistant to digestive enzymes present in saliva and intestine which are only able to cleave α-glycosidic bonds types. Inulins and FOS are degraded in the colon by anaerobic bacteria, however, some limited non-enzymatic acid hydrolysis in the stomach cannot be excluded. The most common dietary sources are wheat, onion, artichoke, garlic and leek. The average intake of inulin has a large variability and is between 3 and 11 g per day in Europe (Van Loo *et al*., 1995). For comparison, the intake in the United States is significantly lower, with a daily consumption average between 1 and 4 g (Moshfegh *et al*., 1999).

The plant that is most commonly used for the industrial extraction of inulin-type fructans belongs to the *Asteraceae* (*Compositae)* family, i.e., chicory (*Cichorium intybus* L.). The native chicory inulin extracted from fresh roots is non-fractionated (De Leenheer, 1996) and to obtain a more effective product, purification procedures and removal of ions are necessary. By applying an endo-inulinase of microbial origin it is possible to obtain, starting from inulin, oligofructose molecules also known as fructooligosaccharides (FOS). FOS are a mixture of GpyFn and FpyFn such as 1-kestotriose, 1,1-kestotetraose and 1,1,1-kestopentaose as well as inulobiose, inulotriose, and inulotetraose all characterized by low DP values ranging between 2 and 7 (Roberfroid, 2007b). FOS might also be produced by the enzymatic process of transfructosylation exerted by a β-fructosidase of *Aspergillus niger* with sucrose as substrate (Shoaf *et al*., 2006). Because of the lower amount of kilocalories found in FOS (1 kcal/g) compared to sucrose (4 kcal/g) (Roberfroid *et al*., 1998) and their higher solubility as compared to inulins, FOS are sometimes used in combination with artificial sweeteners or directly as food additives in yogurt

and derivatives. The use of 8 g of oligofructose per day in 20 healthy subjects has been shown to promote satiety and reduced food intake (Cani *et al*., 2006).

Another important class of prebiotic molecules are the GOS which are mainly obtained by the action of the enzyme β-galactosidase on lactose, resulting in the production of 4'- or 6'-galactosyllactose, longer oligosaccharides, trans-galactosylated disaccharides and non-reducing oligosaccharides consisting of lactose molecules with one or more galactosyl residues linked by β-1,3, β-1,4 and β-1,6 bonds. This variability in glycosidic linkages may be one of the reasons why GOS possess increased resistance to acid digestion (Tomomatsu, 1994). Since birth, the human body gets accustomed to regular intake of GOS. Breast milk provides a variety of GOS based on the lactose, next to the lactose itself (ESPGAN, 1977).

GOS are stable at high temperatures in rather acidic environments and the calorific value of these oligosaccharides is approximately 1 to 7 kcal/g, and therefore they are particularly useful for food applications. They are popular as prebiotics and sweeteners in confectionary, in acidic beverages and in fermented milks. It is validated that the consumption of GOS, like FOS, leads to significant health benefits. For instance, mice infected with *Salmonella enterica serotype Typhimurium* appear to be protected and able to resist the infection when fed with GOS, also enhancing the growth and activity of *Bifidobacterium breve* (Shimizu *et al*., 2001). Recent *in vitro* studies showed that a number of

these substances have the potential to mimic eukaryotic cell surface receptors that are an active part during the pathogenicity process as anchor site for virulent bacteria. For example, GOS was reported to be more efficient than either inulin or FOS in the inhibition of the tight adhesion of EPEC (enteropathogenic *Escherichia coli*) to HEp-2 and Caco-2 cells (Shoaf *et al*., 2006).

Resistant starch

Resistant starch (RS) is defined as "*The sum of the starch and products of starch degradation not absorbed in the small intestine of healthy individuals*" (EURESTA, 1991).

Starch consists of two main components, amylose and amylopectin. Most starches contain mainly amylopectin (often 70-80%) which is an extremely large glucose polymer (10,000-100,000 monomers units) containing α-1,6 branch points. The remaining fraction consists of amylose, a linear polymer (100-10,000 monomer units) of α-1,4 linkages which is mainly found in the amorphous regions of starch granules (Conway, 2001). The starch taken with the diet is not always digested in the upper GI tract. In fact, a large amount is subjected to the process of fermentation in the large intestine (Annison and Topping, 1994; Gibson *et al.*, 2004; Fuentes-Zaragoza *et al*., 2011). The reason for this resistance may be linked to the structural characteristics of the starch granules making them rather inaccessible to the intestinal microflora of the upper part of the gut, besides other factors such as degree of retrogradation after cooking.

Classic plant breeding selection programs led to the production of starch enriched in amylose (80%). This form of high amylose starch from maize

(amylomaize) was shown to improve the SCFA profiles in humans and also functions as a dietary fiber with laxation properties (Noakes *et al.*, 1996; Brown *et al.*, 1997; Topping *et al.*, 1997). A regular administration of amylomaize in diets of pigs reduced the amount of unhealthy microbes such as *Coliforms* (Vaidya and Sheth, 2010). In addition, to prove the effectiveness of these compounds as prebiotics, a screening was performed of more than 40 inhabitants of the human colon. It was demonstrated that only *Bifidobacterium* spp. are capable to use these starch granules as the sole carbon source (Brown *et al.*, 1998). Further studies in mice showed an increase in the levels of *Bifidobacterium* spp. (<log 2 cfu per gram faeces initially to log 8.3 cfu per g feces after four weeks) (Wang *et al.*, 1999a). In some cases it has been shown that *Bifidobacteria* have the feature to use the surface of starch granules, in particular amylomaize, as a site of anchoring. The underlying reason for this behavior is to ensure their protection. In this way, microorganisms become more resistant to pH changes or to excess presence of bile acids. As a consequence, bacterial flora has a higher fitness (Palframan *et al.*, 2002). Other studies now focus on the activity of chemically modified amylomaize starches. The aim of these modifications is to favor the proliferation of *Lactobacilli* both *in vitro* and *in vivo,* to adapt these substances to the desired populations *in situ* (Wang *et al.*, 1999b).

1.1.1.2 Therapeutic use of LDCs

1.1.1.3 Prevention of bacterial infections

Among LDCs, FOS occupy a key position by their ability to change significantly the composition of the colonic microflora, resulting in modulation of host defense and changing interactions between pathogenic and beneficial bacteria.

The gut epithelium is covered by a thick mucus layer that acts as a first defense barrier against the microbiota and pathogenic bacteria and is largely composed of mucin. The mucus layer contains various digestive enzymes and antimicrobial peptides as well as immunoglobulins (Liévin-Le Moal and Servin, 2006; Dharmani *et al*., 2009). The production of mucin is also upregulated as a response to local acidification by regulation of the secretory function of colonic goblet cells (Bertin *et al*., 2001). Higher amounts of mucin, forming gel-like chemical barriers, inhibit colonization and translocation of microbes such as *Salmonella* spp., *Shigella* spp., *Vibrio cholerae*, enteropathogenic *Escherichia coli* (EPEC), enterohemorrhagic *E. coli* (EHEC) and *Yersinia* (Barcelo *et al*., 2000; Bertin *et al*., 2001). EHEC causes illnesses ranging from mild diarrhea to severe diseases such as haemorrhagic colitis and haemolytic uraemic syndrome (Fukuda *et al*., 2011). EHEC (*E. coli* O157) can produce Shiga toxins (Stx), Stx1 and Stx2, which are a crucial factor in lethal infections (Bertin *et al*., 2001). Experimental data show that certain *Bifidobacteria* spp. protect mice against death after *E. coli* infection. This effect can be attributed to increased production of acetate and inhibition of translocation of Stx from the gut lumen into the bloodstream (Fig. 3A) (Ashida *et al*., 2011; Fukuda *et al*., 2011). Acetate improves intestinal defense mediated by epithelial cells and thereby protects the host against lethal infection. SCFAs (especially acetate) can also bind to G protein coupled receptors 41 and 43 on immune cells within the gut-associated lymphoid tissues (GALT) and regulate inflammatory responses (Fig.

3B) (Le Poul *et al*., 2003; Maslowski *et al*., 2009; Ashida *et al*., 2011). It is proposed that high levels of SCFAs may, in addition to its direct effects on the GPR43 response, affect the biosynthesis of endogenous fatty acids, such as resolvins modulating leukocyte functions (Maslowski *et al*., 2009). Butyrate, produced mainly by *Fecalibacterium prausnitzii*, *Eubacterium rectale* and *Roseburia* species, is able to induce the expression of the epithelial antimicrobial peptide LL37 which is correlated with prevention of bacterial infections (Ashida *et al*., 2011). Moreover, it supplies energy for the colonic epithelium and prompts epithelial proliferation as well as injury repair (Guilloteau *et al*., 2010). It has been shown that butyrate can reduce colonic inflammation and bacterial loads in the stool triggered by *Shigella* infection in rabbits (Fig. 3C) (Raqib *et al*., 2006).

Furthermore, it is proposed that LDCs may also influence host immune function by direct interactions with receptors on immune cells, such as T lymphocytes (CD4+ and CD8+) and B lymphocytes (memory cells and plasmocytes) present in GALT. It is postulated that □-glucan carbohydrate moieties can directly bind to the receptor on monocytes/macrophages, neutrophil cell lineages, dendritic cells (DC) and at a lower level on a sub-population of T cells (Herre *et al*., 2004a; Thompson *et al*., 2010). Murine

Dectin-1 receptor and its human homologue hDectin-1 are proposed to be main targets for □-glucan binding and recognition (Taylor *et al*., 2002; Herre *et al*., 2004a,b; Valera *et al*., 2008; Thompson *et al*., 2010). Activation of Dectin-1 initiates endocytosis and phagocytosis, respiratory burst as well as production stimulation of the numerous cytokines and chemokines such as TNF, CXCL2, IL-23, IL-6, IL-10, IL-2, and IL-12 (Taylor *et al*., 2002; Gantner *et al*., 2003; Herre *et al*., 2004a,b,c; Rogers *et al*., 2005; Valera *et al*., 2008; Tsoni and Brown, 2008; Thompson *et al*., 2010). It has been demonstrated that β-glucans have the ability to protect against bacterial, viral, fungal, and protozoal infection and therefore it is often included as an immune booster in feed supplements for farmed animals (Brown and Gordon, 2003; Thompson *et al*., 2010). Recently, also the antiviral activity of fructans was suggested (Esawy *et al*., 2011; Lee *et al*., 2012). As further described in more detail below, both fructans and β-glucans show antitumor activity and they contribute to a lowering of serum cholesterol and glucose levels. Moreover, β-glucans enhance wound repair, influence blood pressure, and support ischemia/reperfusion recovery (Tsoni and Brown, 2008).

SCFAs concentration in different parts of GI tract can directly disturb both viability and virulence gene expression of enteric pathogens species as *Salmonella*, *Pseudomonas aeruginosa* and *Enterobacteriaceae* (Duncan *et al*., 2004; Forchielli and Walker, 2005; Gantois *et al*., 2006; Huang *et al*., 2008; Ashida *et al*., 2011). Differences in concentrations and locations of two particular SCFAs, which are formate and butyrate, induce changes in the intestine environment and can alter virulence characteristics of pathogens like *Salmonella* (Levison, 1973; Cummings *et al*., 1987; Laerke *et al*., 2000; Duncan *et al*., 2004; Forchielli and Walker, 2005; Gantois *et al*., 2006; Huang *et al*., 2008; Ashida *et al*., 2011). For instance, formate is usually present in the

small intestine in low concentrations and is undetectable in the cecum (Gantois *et al*., 2006). In contrast, butyrate is present in the cecum but is not detectable in the small intestine (Gantois *et al*., 2006).

The ability of countless pathogenic microorganisms to adhere to the mucosal surface is crucial for their distribution in the intestine (e.g. *Escherichia coli*, *Helicobacter jejuni*, *Shigella* strains, *Vibrio cholerae*) (Argenzio *et al*., 1974; Miller and McVeagh, 1999; Shoaf *et al*., 2006; Ashida *et al*., 2011). Ligand-receptor interactions between bacterial components and the mucosal surface of the host are essential in the adhesion process. Oligosaccharides and glycoproteins present in large amounts and great variety in the mammals gut can protect from adhesion of microorganisms through their ability to act as receptor analogues (Miller and McVeagh, 1999; Shoaf *et al*., 2006). For example, GOS present in milk and lactulose are capable to constrain adherence of *Campylobacter jejuni*, *E. coli*, *Helicobacter pylori*, and other pathogens (Miller and McVeagh, 1999; Newburg, 1999; Kunz *et al*., 2000; Morrow *et al*., 2005; Shoaf *et al*., 2006). The structures of LDCs are highly associated to their function as it was reported that FOS, inulin, xylo-oligosaccharides (XOS) and their mixtures induce greater inhibition of pathogens than lactulose, lactitol, starch and dextran (Fooks and Gibson, 2002; Ebersbach *et al*., 2012). Furthermore, structural features, such the type of bond and DP affect the

fermentation rate, defining the release rate of inhibitory metabolic end products (Fooks and Gibson, 2002).

Cancer prevention

Colorectal cancer represents a major public health problem causing every year over a half million deaths worldwide (Chau and Cunningham, 2006; WHO, 2012). Treatment highly depends on the stage of the cancer and may include surgery (colectomy), chemotherapy or radiation therapy. Treatment success rates vary for local recurrence, disease-free survival, and overall survival (WHO, 2012). These treatments often present effects such as an increased risk for infections, hair loss, fatigue, vomiting, diarrhea etc. Although threat of colon cancer is upregulated by the genetic factors such as familial polyposis and ulcerative colitis, the main factors include environmental causes such as exposure to carcinogens and dietary composition (Cassidy *et al.*, 1994; Brady *et al.*, 2000; Bruce *et al.*, 2000; Chau and Cunningham, 2006; Ryan-HarshmanAldoori, 2007).

Consumption of fiber and resistant starches is well-thought-out as the protective step in prevention of colon cancer (Cassidy *et al.*, 1994; Chau and Cunningham, 2006; Ryan-Harshman and Aldoori, 2007). Many studies suggest that prebiotics counteract colon carcinogenesis by the production of SCFAs, mainly acetate, propionate and butyrate (Fig. 4) (Topping and Clifton, 2001; Chau and Cunningham, 2006; Liong, 2008). Prebiotics can also modify gene-expression in tumor cells or decrease activity of cancer triggering bacteria (Lorraine *et al.*, 2003).

Dietary carbohydrates recognized in prevention of colon cancer include □-glucans, dietary fibers, fructans and resistant starch (Cassidy *et al*., 1994; Bruce *et al*., 2000; Ryan-Harshman and Aldoori, 2007).

Dietary fibers and □-glucans increase viscosity and bulking in the colon, influencing absorption of minerals, triglycerides and boosting intestinal transit (Scheppach *et al*., 2001; Lorraine *et al*., 2003; Ryan-Harshman and Aldoori, 2007; Grabitske and Slavin, 2008; Liong, 2008). The presence of LDCs in the gut causes a "diluting" effect, reducing the interaction time of potential mutagens and carcinogens with colonic epithelial cells. Furthermore, SCFAs produced through fermentation of LDCs by colonic bacteria decrease the colonic pH, resulting in the growth of beneficial bacteria and depletion of harmful and pathogenic species (Gibson and Wang, 1994; Blaut, 2002; Lorraine *et al*., 2003; Fukuda *et al*., 2011). For instance it is well established that *Clostridium* and *Coliform* infections are associated with the malignancies of the ascending colon (Schaaf *et al*., 1980; Larson *et al*., 1995). Furthermore, the decrease in pH also increases mineral solubility and uptake, specifically calcium, magnesium and iron. Enhanced calcium absorption in the gut restricts calcium depletion from the bones (Lipkin and Newmark, 1995; Lamprecht and Lipkin, 2003; Lim *et al*., 2005). Resistant starches such as high amylose corn starch are highly viscous with properties comparable to a soluble fiber. Their fermentation produces high levels of butyrate which functions at the level of

gene expression, inhibiting malignant transformation by reducing proliferation, and inducing differentiation and cell apoptosis (Bingham, 1990; Kruh *et al.*, 1994; Hague and Paraskeva, 1995; Lorraine *et al.*, 2003). Furthermore, butyrate stimulates the synthesis of proteins such as alkaline phosphatase, glycoproteins, hormone receptors and ion-binding metallothioneins (Scheppach *et al.*, 2001) which may aim to re-establish normal characteristics in developing tumor cells. Evidence exists that butyrate inhibits proliferation of colon cancer cells, initiating an arrest at the early G1 phase due to its action on the gene expression involved in the control of the cell cycle, bearing oncogenes (Toscani *et al.*, 1988; Scheppach *et al.*, 2001).

Inulin-type fructans are well studied in terms of cancer prevention. Significant anticarcinogenic properties have been found in animal studies. Inulin reduced tumor incidence and formation of aberrant crypt foci, initiated by carcinogenic compounds such as azoxymethane (AOM) and dimethylhydrazine (DMH) (Hidaka *et al.*, 1990; Rowland *et al.*, 1998; Pool-Zobel, 2005). Those effects are possibly produced by stimulation of *Bifidobacteria*, which themselves have been shown to act as antigenotoxic in the colon and to reduce AOM-induced tumors.

Protective effects of LDCs rise with increasing structural complexity (DP and branching). For instance, inulin, long-chain inulin and a mixture of short- and long-chain inulin compounds showed a more extended protective effect than oligofructose (Pool-Zobel *et al.*, 2002). This could be attributed to the lower fermentation rate of inulin compared to oligofructose, being able to reach the distal parts of the colon. Inulin-type fructans are known to induce apoptosis of colonic cells with mutations in their DNA. Similarly, elimination of carcinogenic colonic cells by inulin is more effective than oligofructose, again pointing to the importance of structural differences (Hughes and

Rowland, 2001). Inulin-type fructans are fermented extensively by large bowel microflora to lactic acid and SCFAs (Hidaka *et al.*, 1990; Pool-Zobel *et al.*, 2002) contributing to the protective effects and apoptosis induction.

Permeability of inulin has been measured in normal, adenomatous, colitic and malignant large bowel epithelial cells showing that all carcinomas displayed its extensive uptake. Accumulation of inulin by carcinomas could not only be attributed to pinocytosis (Chambers and Serafini, 1985) since only partial inhibition was observed with cytochalasin B, a well- known inhibitor of pinocytosis and phagocytosis (Von Figura and Kresse, 1974). These data suggested the existence of alternative uptake mechanisms besides pinocytosis (North, 1983). Compared to carcinomas, normal, adenomatous and colitic epithelial seem to have a more limited permeability to inulin, which is correlated with its molecular weight and hydrodynamic radius (Ghandehari *et al.*, 1997). This suggested that membrane properties, vastly depending on their lipid and protein composition, may be an important factor explaining the extended inulin uptake in carcinogenic cells. The increased membrane fluidity of cancer cells is associated with metastasis (Nakazawa and Iwaizumi, 1989; Sok *et al.*, 2002; Wang, 2005) and often specific characteristics are observed such as the inability to synthesize some types of lipids (as for example glycolipids), or the deletion of terminal saccharide residues in glycolipids (Wang, 2005). Increase in ion permeability can induce extensive proliferation

and compromised reception of differentiation directing signals from neighboring cell due to closure of gap junctions (Chambers and Serafini, 1985). It is postulated that inulin accumulation can slow down tumor proliferation and induce apoptosis (*Munjal et al*., 2009; Chung *et al*., 2011) although further studies are necessary to unravel the precise action mechanism.

The role of reactive oxygen species (ROS) in carcinogenesis has been extensively investigated during the last decade. ROS play an important role in the modulation of several physiologic responses as induction of programmed cell death or necrosis, induction or suppression of genes expression, activation of cell signaling cascades and induction or inhibition of cell proliferation (Hancock *et al*., 2001; Gibellini *et al*., 2010). ROS, if produced in an uncontrolled manner, induce cellular damage and can give rise to pathological conditions. One of the key features of the cancer cells is a tenacious pro-oxidative state leading to oxidative stress (Szatrowski and Nathan, 1991; Toyokuni *et al*., 1995; Gibellini *et al*., 2010). ROS are continuously created in cancer cells as a result of several factors, including increased metabolic activity, the activation of oncogenes, and the eventual loss of tumor suppressor protein p53 (Pelicano *et al*., 2004; Gibellini *et al*., 2010).

Flavonoids -compounds naturally occurring in a great variety of foods and beverages- are believed to counteract carcinogenesis, mainly due to their antioxidant properties (Gibellini *et al*., 2010). They have also the ability to modify proteins involved in cell proliferation and cell death pathways (Gibellini *et al*., 2010). Quercetin (Qu) is an important dietary flavonoid, present in different vegetables, fruits, seeds, nuts, tea, and red wine. Quercetin can affect ROS metabolism and induce cell apoptosis (Gibellini *et al*., 2010). When cancer cells, containing high levels of ROS, are exposed to quercetin, toxic oxidation products are formed (quercetin-semiquinones and quercetin-quinones;

Fig. 5) (Metodiewa *et al*., 1999; Gibellini *et al*., 2010). Those chemicals exert pro-oxidant properties and are highly reactive towards thiols and easily react with reduced glutathione (GSH) initiating its depletion. In normal cells exposed to ROS, as for instance H_2O_2, GSH biosynthetic pathways are stimulated to counteract the oxidative stress (Ferraresi *et al*., 2005; Gibellini *et al*., 2010). ROS increases play an important role in the manifestation and conservation of the cancer phenotype, leading to a pro-oxidative state. ROS induce chromosomal instability through accumulation of mutations and deletions in the genetic material and stimulates cell growth and proliferation, as well as cell migration and invasiveness (angiogenesis and metastasis). The adaptation of cancer cells to high levels of ROS involves modification of the antioxidant functions and upregulation of pro-survival proteins which allows them to escape apoptosis (Fig. 5) (Gibellini *et al*., 2010).

Quercetin triggers GSH depletion, eliciting apoptosis via mitochondrial depolarization (Ferraresi *et al*., 2005; Lugli *et al*., 2005; Ly *et al*., 2003; Choi *et al*., 2005; Troiano *et al*., 2007; Gibellini *et al*., 2010). Moreover, when levels of GSH are highly reduced, products of mitochondrial pathways such as H_2O_2 can damage the organelle through loss of mitochondrial membrane potential and release of cytochrome *c* into the cytosol (Fig. 6). Finally activation of caspases occurs, such as caspase-3 and caspase-7, and apoptosis takes place (Kuo *et al*.,

2004; Choi *et al*., 2005; Yang *et al*., 2006; Chien *et al*., 2009; Chou *et al*., 2010; Niue *et al*., 2011).

Quercetin can also modulate pro-apoptotic and antiapoptotic proteins such as Bax, Bak, Bcl-2 and Bcl-xL (Kuo *et al*., 2004; Yang *et al*., 2006; Chien *et al*., 2009; Niu *et al*., 2011) and the PI_3K/Akt pathway, which are important in cell survival and proliferation (Yang *et al*., 2006; Gibellini *et al*., 2010).

Further studies are needed on the exact uptake mechanisms of flavonoids and prebiotics, on the exact concentrations of these compounds in epithelial cells and on the interactions between these compounds, ROS and cellular proteins. It can be speculated that these compounds act as direct ROS scavengers (Bolouri-Moghaddam *et al.*, 2010; Van den Ende *et al*., 2011). Alternatively, and perhaps more likely, they can interfere with the uptake mechanisms of glucose or other metabolites and/or with AMPK signaling pathways (De Gara *et al*., 2003; Yun *et al*., 2009; Stoyanova *et al*., 2011). In particular, the antioxidant effect of levan-type fructans under oxidative stress conditions was examined on pancreatic INS-1E cells. Under high glucose condition, oxidative stress and apoptosis were significantly increased in cells treated with hydrogen peroxide. However, treatment with levan decreased oxidative stress and attenuated apoptosis (Kazak *et al*., 2011).

Diabetes and metabolic syndrome prevention

Metabolic syndrome is a collection of conditions that as a group increases the risk of developing cardiovascular disease and type 2 diabetes mellitus. These disorders include high blood pressure, high sugar and triglycerides levels. Central obesity and insulin resistance are recognized as significant activating factors. Other causes include physical inactivity, ageing and hormonal

imbalance caused by polycystic ovary syndrome (PCOS) or testosterone insufficiency (Anderson *et al*., 2001; Gustat *et al*., 2002; Cho, 2011).

Diabetes mellitus is typically characterized by high blood glucose levels, resulting from defects in insulin secretion or insulin insensitivity. Diabetes is a chronic medical illness that can produce coronary artery disease, cerebrovascular disease, renal failure, limb amputation, blindness, neurological complications and premature death (Weidmann *et al*., 1993; Gobinath *et al*., 2010).

In animal model studies, dietary supplementation of 10% XOS and FOS were able to improve body weight in diabetic rats, reduced mortality and significantly increased the *Bifidobacteria* and *Lactobacilli* population in the caecum (Gobinath *et al*., 2010). The improved bacterial population induced a reduction of the pH in caeca associated with rising SCFA concentrations. Lactate, acetate, propionate and butyrate, which are rapidly absorbed from the lumen of the colon, can influence glucose levels, lipid metabolism and lower cholesterol levels (Venter *et al*., 1990; Laurent *et al*., 1995; Al-Lahham *et al*., 2010; Gobinath *et al*., 2010). Acetate passes to peripheral tissues where it is metabolized by muscles, adipose tissue or heart tissue (Mayfield *et al*., 1966). Butyrate is largely utilized by the colonocytes and propionate is removed by the liver (Cummings *et al*., 1987; Gobinath *et al*., 2010) influencing the intermediary metabolism in those tissues.

Further examination of butyric acid metabolic activities in diet-induced obese mice demonstrated that butyrate supplementation at 5% wt/wt in high-fat diet prevented development of dietary obesity and insulin resistance (Fig. 7A). The change in insulin sensitivity may be a consequence of a reduction in adipose tissue levels (Gao *et al.*, 2009; Gobinath *et al.*, 2010). This may suggest that butyrate could be an effective compound in the treatment of obesity and insulin resistance in obese individuals.

Furthermore, plasma glucose level could be significantly decreased in diabetic rats in response to administration of XOS or FOS. Intake of FOS has been reported to decrease fasting blood glucose levels in diabetic subjects and dietary supplementation with XOS improved blood sugar and lipids levels in type 2 diabetes in murine (Fig. 7B) and human models (Luo *et al.*, 2000; Sheu *et al.*, 2008; Gao *et al.*, 2009). It can be concluded that consumption of FOS- or XOS-containing diets could be beneficial in relieving severity of hyperglycaemia.

It is well established that elevated plasma LDL-cholesterol concentrations can increase risk on heart diseases. Many studies confirm that dietary supplementation with LDCs- effectively reduce plasma cholesterol levels. The mechanism underlying the cholesterol lowering effects of SCFA such as propionate, includes the inhibition of cholesterol synthesizing enzyme (3-hydroxy 3-methylglutaryl CoA reductase) and redistribution of cholesterol from the blood plasma to the liver (Wright *et al.*, 1990; Arora *et al.*, 2011). Propionate can also improve the secretion and synthesis of bile acids, and cholesterol 7α hydroxylase activity in primary cultured rat hepatocytes by increasing mitochondrial succinyl CoA concentrations (Imaizumi *et al.*, 1992; Levrat *et al.*, 1994). Furthermore, addition of propionate and butyrate to Caco-2

cell lines trigger downregulation of genes involved in the intestinal cholesterol synthesis pathway (Alvaro *et al*., 2008).

Carbohydrates such as inulin, resistant starch and l-rhamnose lead to increased concentrations of propionate after fermentation, exhibiting hypocholesterolemic effects by reduction of serum total cholesterol levels and hepatic triglyceride levels both in animals and humans (Trautwein *et al*., 1998; Cheng and Lai, 2000; Vogt *et al*., 2004).

Moreover, high LDC intake is associated with lowering blood pressure and prevention of cardiovascular diseases (Fernandez, 2001; Yeo *et al*., 2009). A number of mechanisms have been proposed to clarify the action in prevention of hypertension and heart diseases. Lowering of blood lipid and cholesterol levels, reduction of obesity, reduction of diabetes risk and improving absorption of minerals might be important in this respect (Yeo *et al*., 2009).

The group of soluble prebiotics to which belongs pectin, konjac mannan, guar gum, xanthan gum and modified starches cause a thickening and viscous effect in solutions (Levrat-Verny *et al*., 2000). Those properties are highly beneficial in lowering blood cholesterol levels and for increasing satiety. Further, insoluble grain fibers can be associated with reduced risk on diabetes, because they are fermented to SCFAs which improve hepatic insulin sensitivity (Weickert *et al*., 2006; Yeo *et al*., 2009). As mentioned previously, prebiotics improve the absorption of minerals as calcium which can be associated with a

reduction of hypertension (Allender *et al*., 1996; Zemel, 2001). Also inulin has been reported to enhance the absorption of calcium in human (Abrams *et al*., 2005; Yeo *et al*., 2009). Prebiotics increase the absorption of calcium mainly due to sequestering it in the gastrointestinal tract. When reaching the colon, calcium is released from the prebiotic matrix and absorbed in epithelial cells (Scholz-Ahrens *et al*., 2001; Yeo *et al*., 2009).

Gastrointestinal diseases treatment and prevention

Digestive diseases comprise of many acute and chronic disorders of the GI tract ranging from common digestive syndromes as constipation to serious and lethal diseases. GI illnesses include functional bowel diseases such as irritable bowel syndrome (IBS) and inflammatory bowel diseases (IBD) such as Crohn's disease (CD) and ulcerative colitis (UC). Many GI disorders can be relieved by diet and medications, but a large majority of them are difficult to treat by conventional medicine. Symptoms of GI disorders often include cramping, abdominal pain, inflammation of the lining of the large and/or small intestine, chronic diarrhea, rectal bleeding and weight loss (Leenen and Dieleman, 2007). Inflammatory bowel disease represents the clinical outcome of three interactive pathogenic factors: genetic susceptibility, environmental triggers and immune deregulation (Shanahan, 2004).

CD can occur at any location in the intestinal tract with diverse symptoms involving chronic inflammation of the gastrointestinal tract, non-bloody diarrhea, abdominal cramps, fever, weight loss, and perianal manifestations (Leenen and Dieleman, 2007). Treatments for CD include pharmaceuticals as steroids, 5-aminosalicylic acid, antibiotics, azathioprine/6-mercaptopurine and methotrexate (Leenen and Dieleman, 2007).

In CD, nucleotide-binding oligomerization domain-containing protein 2 (NOD2) gene mutations, are allied with decreased transcription of the anti-inflammatory cytokine interleukin 10 (IL-10). A reduction in IL-10 levels contributes to magnification of the granulomatous reaction and lack of resolution of inflammation, leading to a pathogenic state (Fava and Danese, 2010). Furthermore, NOD2 expression is involved in regulation of the gut wall colonization by bacteria with higher levels of bacterial colonization in Nod2-deficient animals and as well in human (Fava and Danese, 2010). The intestinal microbiota play a vital role in the inflammation associated with CD disease especially including immunoregulatory *Bifidobacteria* species interacting with the mucosal immune system. They induce dendritic cells to produce and release high amounts of IL-10 (Hart *et al*., 2004; Sartor, 2004). Prebiotics such as FOS, XOS or inulin, with their capacity to increase fecal and mucosal *Bifidobacteria,* can modulate mucosal dendritic cell (DC) function in patients with CD (Lindsay et al., 2006; Leenen and Dieleman, 2007; Hedin *et al.*, 2007). Moreover, DCs expressed higher levels of Toll-like receptor 2 (TLR2) and/or TLR4 receptors following FOS supplementation. Upregulation of TLR expression is a possible result of better recognition of *Bifidobacteria* by DC and signaling via TLRs which is often correlated with initiation of inflammatory responses (Hausmann *et al*., 2000). Nevertheless, recent studies proposed a perspective shielding role for TLRs in the gut. TLR activation is indispensable

for intestinal homeostasis, protection against gut injury and epithelial restitution (Hausmann *et al*., 2000; Rakoff-Nahoum *et al*., 2004). Although the mechanism by which prebiotics regulate expression of TLR expression in DCs remains to be determined, their beneficial role in treating inflammatory bowel diseases is clear.

UC is a colon inflammation and involves symptoms as diarrhea, rectal bleeding, and abdominal pain, often accompanied by fever and weight loss. Pseudopolyps, which are commonly detected during endoscopy of individuals with UC, increase the risk on developing colorectal cancer at later stages. Medical treatment of UC is vastly related to CD treatment. Prebiotics as inulin, oligofructose-enriched inulin and goat milk oligosaccharides are known to reduce UC symptoms in murine models (Videla *et al*., 2001; Hoentjen *et al*., 2005; Daddaoua *et al*., 2006; Lara-Villoslada *et al*., 2006). Those LDCs inhibit the adhesion of bacteria to the epithelial membrane, reduce bacterial translocation and promote selective growth of Lactobacilli and *Bifidobacteria* (Videla *et al*., 2001; Hoentjen *et al*., 2005; Daddaoua *et al*., 2006; Lara-Villoslada *et al*., 2006). At present LDCs are often recommended as valuable alternatives to current therapies to treat IBD. Although inulin-type fructans and FOS showed a beneficial role in IBS symptome reduction in many individuals, the opposite effects may arise depending on patient sensitivity, supplementation periods and doses (Kelly, 2009). For example oligofructoses, inulin and GOS, which are able to increase stool weight and relieve constipation, can also increase flatulence and bloating (Spiller, 2008; Kelly, 2009). Also supplementation with lactulose, one of the best recognized treatments of constipation, is not fully beneficial due to production of significant amounts of gas and abdominal pain that aggravate symptoms of IBS (Spiller, 2008). Compounds that are poorly absorbed in the intestine such as sorbitol, fructose

and polyhydric alcohols are used as substrates for bacterial fermentation (Fernandez-Bañares *et al*., 1991; Symons *et al*., 1992; Spiller, 2008). Due to their small molecular weights they trap a considerable amount of water in the gut causing unwanted diarrhea in some patients (Fernandez-Bañares *et al*., 1991; Symons *et al*., 1992). As a consequence, the use of prebiotics to treat IBS is still uncertain and awaits more trials.

Strategies to increase prebiotics properties

As discussed above, prebiotics are highly important natural compounds that could be involved in treatment and prevention of many diseases. The improvement of the prebiotic activity is an essential pharmaceutical target and follows two main strategies. The first is to seek new molecules with a potential superior prebiotic activity. The recent success of the concept of prebiotics and functional foods has stimulated researchers to examine, through a screening, the possibility of identifying other molecules with similar or even better properties. Among the most explored groups are the starch-polysaccharides and â-glucans. Supplementation of rodent diets with 1% â-glucans of yeast origin increased defenses against *Salmonella typhimurium* and enhanced the level of fecal *Lactobacilli* and *Bifidobacteria* (Ho Hoa, 2000).

Other potential prebiotics are acacia gums. Research demonstrated that, after administration of two of these compounds, the levels of SCFAs were increased. At the same time an inhibition of *Clostridium* was recorded, in both cases, and an active stimulation of *Lactobacilli* in one case (Michel *et al.*, 1998).

Another improvement strategy is focused on "whole colon" protection. Through the use of mixtures containing different types of prebiotics with different structural features (DP and branching), one attempts to establish fermentation processes along the entire surface of the colon. It is well-known that small, linear oligosaccharides are more quickly utilized in the proximal part of the colon while, on the contrary, the resistant starch, branched or higher DP saccharides are degraded more slowly, at least partially reaching the distal part of the colon. For example, the effects of a diet based on wheat bran and resistant starch on pigs were studied and indeed, the presence of wheat bran caused an increased concentration of butyrate in the distal portion of the colon (Govers *et al.*, 1999).

For the same reasons, attention is shifting from inulin-type fructans to the use of alternative type fructans (e.g., wheat graminans and Agave fructans) as promising prebiotics (Huazano-Garcia *et al.*, 2009; Gomez *et al.*, 2009; Casiraghi *et al.*, 2011; Jenkins *et al.*, 2011). Finally, some prebiotics seem to selectively stimulate probiotic strains. This was recently reported for a 2-substituted-(1,3)β-D-glucan. Moreover, the presence of this particular glucan stimulated the *in vitro* adhesion of the probiotic *Lactobacillus plantarum* WCFS1 to human intestinal epithelial cells (Russo *et al.*, 2012).

Concluding remarks

Technological and scientific progress has allowed us to understand the underlying mechanisms of diseases, their treatment and prevention. Functional foods are important class of molecules which open new perspectives in research over cure of many disorders. The basic concept considered and analyzed in this review is that the inception of chronic degenerative diseases of gastrointestinal tract and other parts of our body might be prevented by the daily application of a diet based on specific foods from the beneficial group of prebiotics. The profits of prebiotics consumption have been known for a long time, but currently the main aim is to focus on their role in the prevention mechanism underlying the onset of diseases such as colon cancer, diabetes mellitus, obesity and also on the symbiotic interaction among human organism and the microbial flora. The extensive evidence presented in this review, based on the work of many research groups on animals and human, strongly suggest that prebiotics are strong candidates in the area of disease prevention. A diet rich in prebiotics has been proven to protect from many illnesses. Therefore, it can be concluded that their action is not fiction but reality. However, large scale clinical studies are necessary to further corroborate these findings.

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Figure legends

Figure 1. Functions of the intestinal microflora (A) Changes in the bacterial populations in the different parts of the GI tract. (B) Main aerobic and anaerobic species found in healthy individuals which exert a combination of

protective, structural and metabolic effects on the intestinal mucosa (Figures from O'Hara *et al.*, 2006).

Figure 2. An overview of plant fructan synthesis starting from sucrose. Inulin-, levan-, graminan-, neo-inulin and neo-levan type fructans can be discriminated. The following enzymes are involved: sucrose:sucrose 1-fructosyl transferase (1-SST), sucrose:fructan 6-fructosyl transferase (6-SFT), fructan:fructan 6G-fructosyl transferase (6G-FFT) and fructan:fructan 1-fructosyl transferase (1-FFT).

Figure 3. Role of SCFAs as important elements preventing colonization of bacterial pathogens. (A) Acetate produced by *Bifidobacterium* origins acidification and prevents colonization by EHEC and also prevents translocation of Shiga toxin (Stx2) into the bloodstream. (B) Direct binding of acetate to G protein coupled receptors 43 (GPR43) on immune cells regulates inflammatory responses. (C) Butyrate prevents infection by up-regulation of antimicrobial peptides (Figures adapted from Ashida *et al.*, 2011).

Figure 4. Beneficial effects of short-chain fatty acids on cells in various stages of carcinogenesis**.** But (n-butyrate), Prop (propionate), Ac (acetate) (Figure modified from Scheppach *et al.*, 2001).

Figure 5. The role of quercetin in cancer defence. LPO (lactic peroxidase); O_2^- (superoxide anion); H_2O_2 (hydrogen peroxide); GSH (glutathione); GS• (oxidized GSH); GSSG (glutathione disulfide) (Figure derived from Gibellini *et al.*, 2010).

Figure 6. Cellular effects induced by cytoprotective flavonoids. The → designates activation or induction, and ⊣ inhibition or blockade. Bcl-2 (B-cell lymphoma 2), Bax (Bcl-associated X protein), Caspase-3 (cysteine-aspartic protease 3), p53 (tumor protein 53) (Figure modified from Choi *et al.*, 2005).

Figure 7. Treatment of obesity and high glucose levels in mice with use of butyrate. Obesity was induced in C57BL/6J mice fed a high-fat diet for 16 weeks (21 weeks in age). The obese mice were then treated with butyrate

through food supplementation for 5 weeks. (A) In control mice increase of body weight (BW) has been measured after 5 weeks of fed with a high-fat diet. Mice which receive a high-fat diet with 5% wt/wt butyrate reduced weight during same treatment time. (B) Fasting glucose levels ware reduced in group treated with a high-fat diet with 5% wt/wt butyrate supplementation. Tail vein blood was used for glucose assay after 16 h fasting during the period of high-fat diet feeding. Values are the means ± SE (n = 8 in each group). *P < 0.05. **P < 0.001 (n = 2) (Data used from Gao *et al.*, 2009).

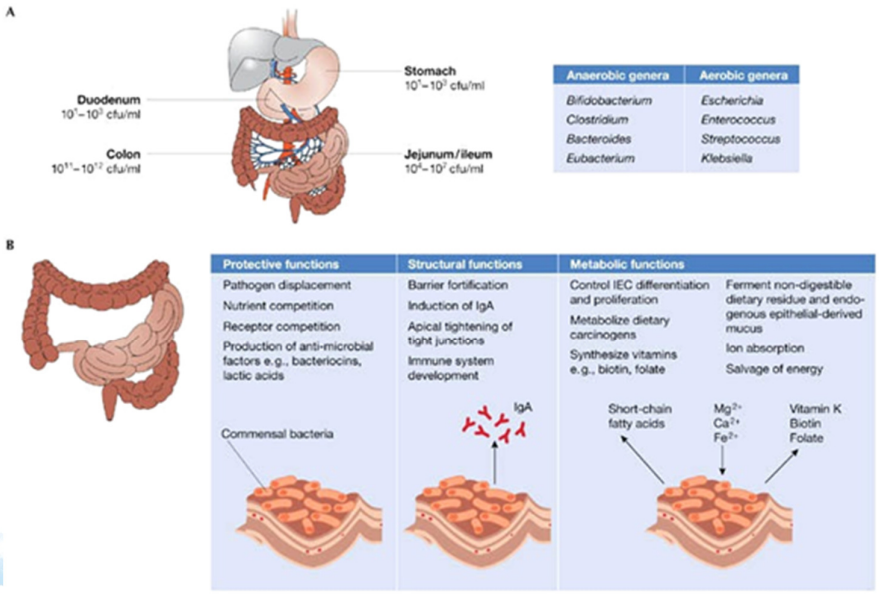

Fig. 1

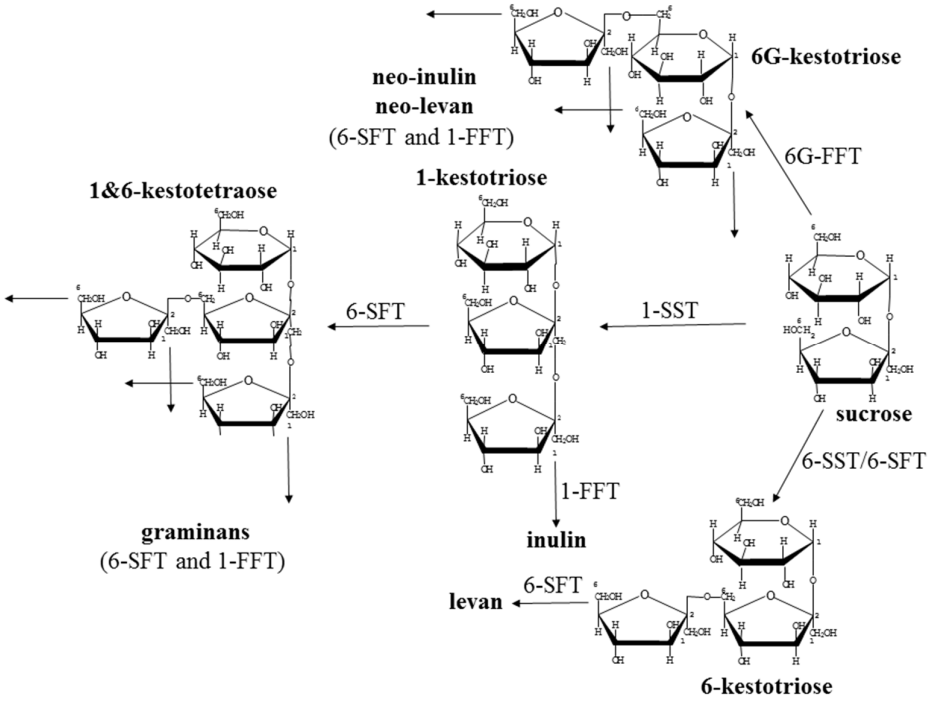
neo-inulin
neo-levan
(6-SFT and 1-FFT)
6G-kestotriose
6G-FFT
1-kestotriose
1&6-kestotetraose
6-SFT
1-SST
sucrose
6-SST/6-SFT
1-FFT
graminans
(6-SFT and 1-FFT)
inulin
6-SFT
levan
6-kestotriose

Fig. 2

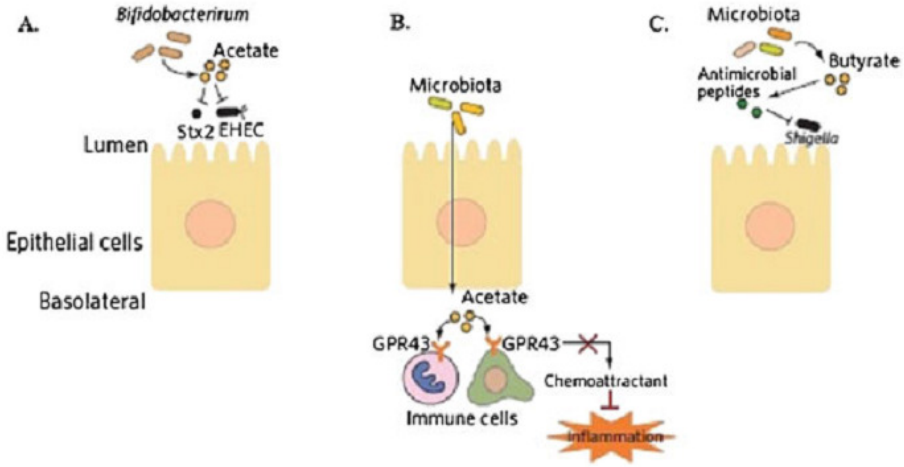
A.
Bifidobacterirum
Acetate
Stx2 EHEC
Lumen
Epithelial cells
Basolateral
B.
Microbiota
Acetate
GPR43
GPR43
Chemoattractant
Immune cells
Inflammation
C.
Microbiota
Butyrate
Antimicrobial peptides
Shigella

Fig. 3

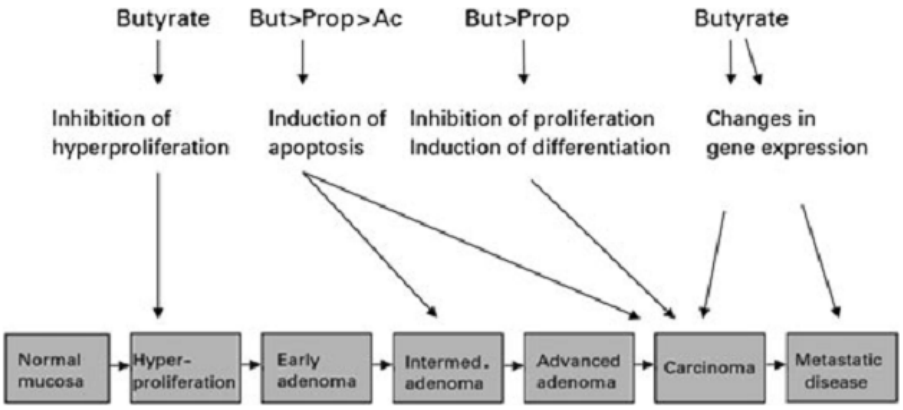
Butyrate
But>Prop>Ac
But>Prop
Butyrate
Inhibition of hyperproliferation
Induction of apoptosis
Inhibition of proliferation
Induction of differentiation
Changes in gene expression
Normal mucosa
Hyper-proliferation
Early adenoma
Intermed. adenoma
Advanced adenoma
Carcinoma
Metastatic disease

Fig. 4

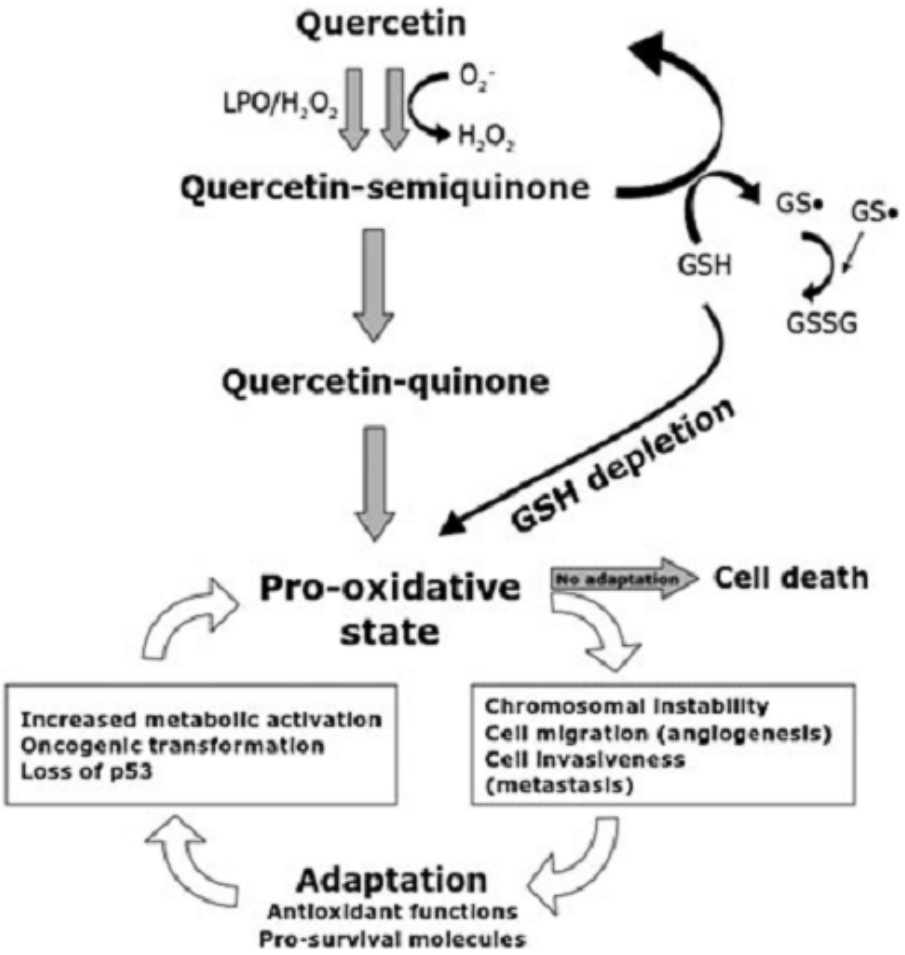
Quercetin
LPO/H2O2
O2-
H2O2
Quercetin-semiquinone
GS•
GS•
GSH
GSSG
Quercetin-quinone
GSH depletion
Pro-oxidative state
No adaptation
Cell death
Increased metabolic activation
Oncogenic transformation
Loss of p53
Chromosomal instability
Cell migration (angiogenesis)
Cell invasiveness
(metastasis)
Adaptation
Antioxidant functions
Pro-survival molecules

Fig. 5

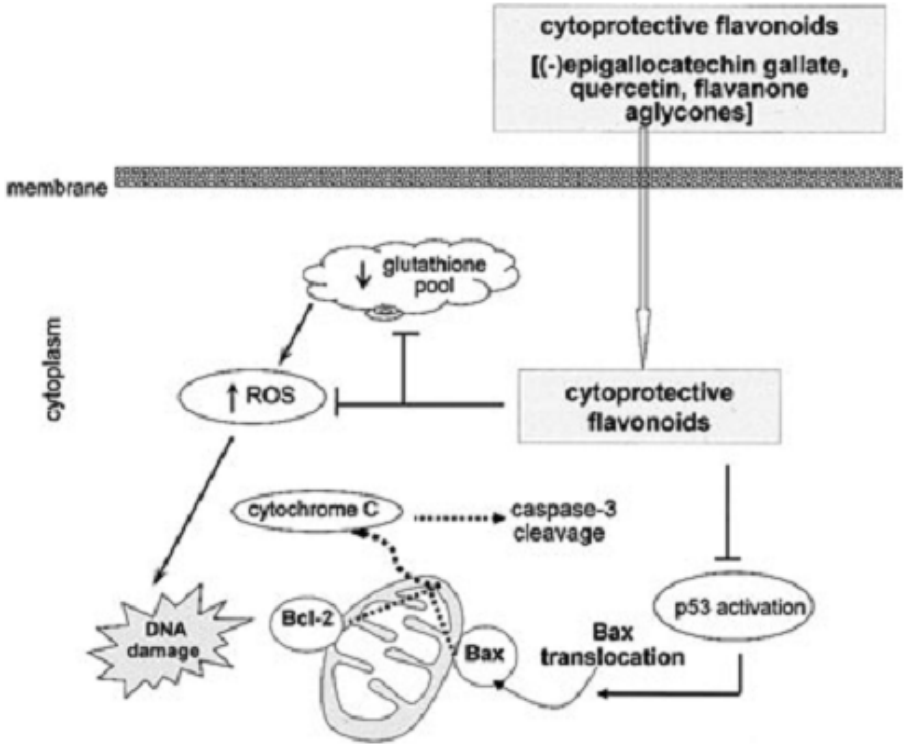
cytoprotective flavonoids
[(-)epigallocatechin gallate, quercetin, flavanone aglycones]
membrane
glutathione pool
cytoplasm
ROS
cytoprotective flavonoids
cytochrome C
caspase-3 cleavage
DNA damage
Bcl-2
Bax
Bax translocation
p53 activation

Fig. 6

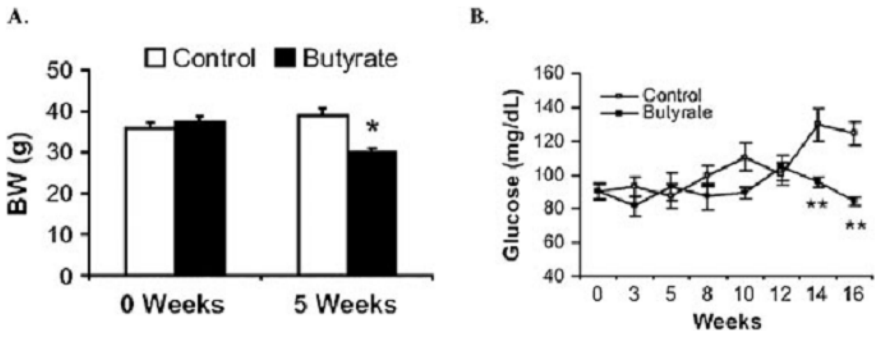
A.
Control
Butyrate
BW (g)
50
40
30
20
10
0
*
0 Weeks
5 Weeks
B.
Glucose (mg/dL)
160
140
120
100
80
60
40
Control
Bulyrate
**
**
0 3 5 8 10 12 14 16
Weeks

Fig. 7